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13. ABSTRACT *(Maximum 200 words)*

The following findings were reported: 1)Oxotremorine-M binding in rabbit thalamus & cingulate cortex increased during discriminative avoidance conditioning (DAC). This is the first report of behavioral regulation of muscarinic receptors. 2) Excitatory & discriminative neuronal activity was documented throughout DAC and there were relationships between training-induced neuronal activity and changes in binding. 3) Turnover of noradrenaline was significantly elevated during DAC suggesting a role for this transmitter in long-term memory. 4) Anterior cingulate cortex lesions uncover discriminative neuronal activity in the striatum and amplify activity in thalamus 5) The structure connections and spontaneous activity of the lateral magnocellular nucleus in thalamus were described. 6) A review was written of the structure and function of cortical layer I and its role in learning and memory analyzed. 7) A volume titled "Organization and Functions of Cingulate Cortex and Limbic Thalamus" was organized and its publication will be due largely to AFOSR support to the editors.

These are the first studies to document physiological regulation of receptors and transmitters that occur during avoidance learning and provide the basis for a comprehensive analysis of the molecular bases for learning and memory.

14. SUBJECT TERMS
muscarinic acetylcholine receptors; discriminative avoidance learning, training-induced neuronal activity; noradrenaline; cortical layer I; molecular bases of learning and memory

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FINAL TECHNICAL REPORT

Receptor Subtype Alterations: Bases of Neuronal Plasticity and Learning

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1990

SUMMARY

During two years at Boston University the following projects were completed: 1) It was shown that oxotremorine-M binding in rabbit anterior thalamus and cingulate cortex increased during the course of discriminative avoidance conditioning (DAC). Since there was no change in pirenzepine binding, it was suggested that this change was associated with muscarinic (M2) receptors. 2) Excitatory and discriminative neuronal activity was documented in 5 thalamic nuclei and posterior cingulate cortex throughout DAC. There were striking relationships in the anterior thalamus between training-induced neuronal plasticities and changes in oxotremorine-M binding. 3) The concentrations of noradrenaline, serotonin and dopamine and their principal metabolites were measured in 5 rostrocaudal levels of cingulate cortex with HPLC and coulometric detection in animals that were trained to different levels of behavioral performance. Noradrenaline turnover was significantly elevated during DAC. 4) Anterior cingulate cortex lesions were shown to uncover discriminative neuronal activity in the striatum and amplify neuronal activity in mediodorsal thalamus. 5) The structure, connections and spontaneous activity of neurons in the lateral magnocellular nucleus in rabbit thalamus were described. 6) A review was written of the structure and function of cortical layer I and its role in learning and memory. 7) A volume titled "Organization and Functions of Cingulate Cortex and Limbic Thalamus" was organized and its publication will be due largely to AFOSR support to the editors. 8) Apparatus with computer control was built for DAC. 9) The Boston laboratory was dismantled and moved to Bowman Gray School of Medicine.

RESEARCH OBJECTIVES

There were four goals of the original application which sought to 1) specify behavioral stage-related alterations in muscarinic acetylcholine and GABA receptor subtype binding in cingulate cortex and limbic thalamus that occur during the acquisition and performance of an active avoidance task, 2) determine which neurons have increased muscarinic receptor binding, 3) investigate which cingulate cortical afferents to thalamus trigger such alterations in binding and 4) analyze the role of cingulate afferents to thalamus in stage-related alterations in thalamic receptor subtype binding. Receptor subtype binding to cortical layers and thalamic nuclei was to be evaluated in cryostat sections with coverslip autoradiography and single grain counting techniques. Ligand binding protocols included the following: M_1, ^{3}H-pirenzepine; M_2, ^{3}H-oxotremorine-M in the presence of unlabeled pirenzepine; $GABA_A$, ^{3}H-muscimol; M_1 and M_2 in dissociated neurons, ^{3}H-propylbenzilylcholine mustard.

Three experiments were proposed to accomplish the above stated goals. **First,** alterations in binding to muscarinic and GABA receptors at different stages of active avoidance learning were to be analyzed in cingulate cortex and limbic thalamus. The stages included naive, pretraining, first exposure to paired conditional and unconditional stimuli, first significant behavioral response, criterial performance, overtraining and animals that were yoked to criterial performance. **Second,** neurons involved in up regulation of M_1 receptors and afferents triggering these events were to be analyzed with dissociated neuron and deafferentation lesions. The latter lesions were to be placed in the diagonal band of Broca, limbic thalamus or subiculum. **Third,** lesions of the lateral dorsal tegmental nucleus were to be made followed by behavioral training and muscarinic receptor binding assay.

STATUS OF THE RESEARCH

I. Prologue

This research was proposed as a collaboration between the P.I. and Dr. Michael Gabriel at the University of Illinois. The proposed projects were particularly unique because they united two established programs in receptor pharmacology and behavioral neurophysiology. We are pleased to report that this collaboration has provided important new findings about alterations in receptor binding which occur during discriminative avoidance learning. In the renewal application which each investigator will be submitting, it is proposed that a joint project be funded in order to continue this productive relationship.

The original proposal of the P.I. was funded at Boston University School of Medicine. The first two years of this research are evaluated in this Final Technical Report. Since the P.I. has moved to Bowman Gray School of Medicine, the third year of this application was awarded as a new application to the Bowman Gray School of Medicine. Therefore, a Final Technical Report will be submitted from Bowman Gray School of Medicine for this third year.

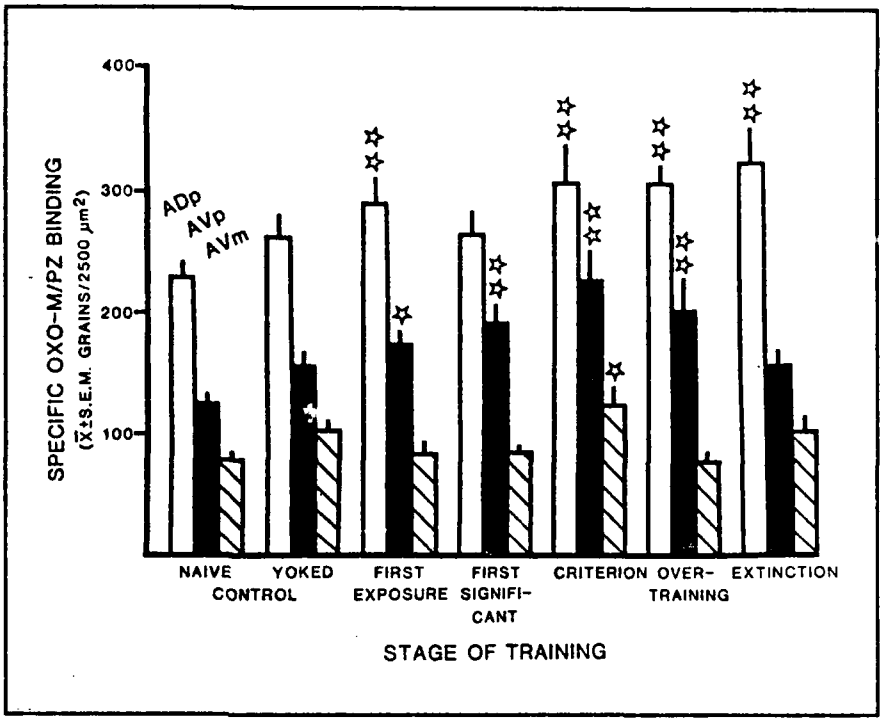
The original proposal was based on a pilot study of training-induced changes in receptor binding in 14 rabbits (4 naive control and 10 behaviorally-conditioned animals). During the first year of funding we explored our tentative conclusions with further testing. These findings led us to alter our goals in a number of important ways. **First**, the increase in pirenzepine binding in the pilot study could not be confirmed and so we have not pursued the neuron dissociation experiment. This latter experiment was intended to explore changes in binding on cortical neuron dendrites and was not necessary if we could not consistently demonstrate changes in binding to postsynaptic receptors. **Second**, there were consistent changes in binding of oxotremorine-M to what is most likely a presynaptic, M_2 receptor. These studies required many more cases than we had originally planned to prepare; 82 in all. In addition, we developed ligand binding protocols for many other receptors and analyzed binding in these cases to determine if other transmitter systems might also be involved. Processing many more cases and ligands meant that we were not able to fully explore the consequences of deafferentation lesions during this grant period. **Third**, we expect that it will require at least three complete sets of data to explore the influences of lateral dorsal tegmental and mammillary body lesions on alterations in muscarinic and GABA receptor binding in the thalamus. The first of these experiments will be done during the third year of funding. The second and third experiments and most data analysis will be pursued during the first two years of the proposed renewal period. **Finally**, samples from many of these brains were analyzed with a 16 coulometric electrode system in conjunction with high pressure liquid chromatography. This study was not originally proposed, but it has shown an important involvement of noradrenaline in the acquisition of this task.

II. Progress Toward Research Objectives

A. Alterations in Muscarinic Receptor Binding

Vogt, Gabriel, Vogt, Poremba, Jensen, Kubota and Kang (1991) reported for the first time that ^{3}H-oxotremorine-M binding in the presence of unlabeled pirenzepine (OXO-M/PZ)

Figure 1

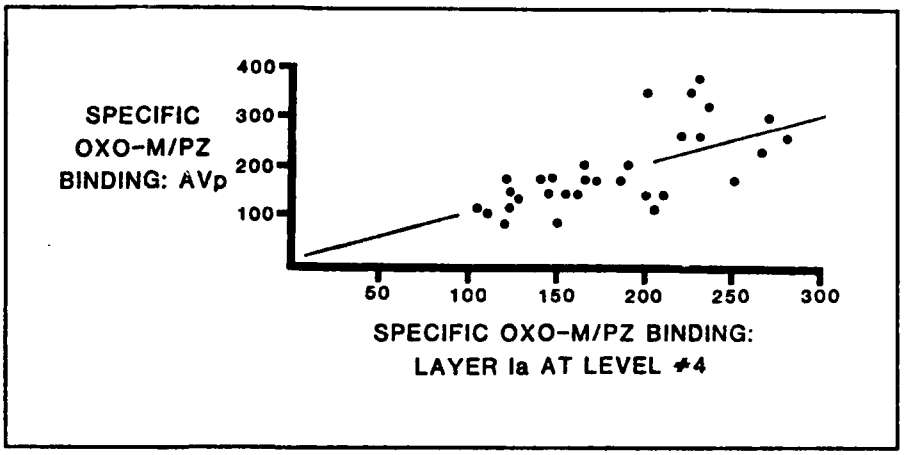


was increased in the anterior thalamus and cingulate cortex of rabbits throughout the course of discriminative avoidance training. Upon reaching a particular stage of training the brains were removed and autoradiographically assayed for oxotremorine-M, pirenzepine, muscimol, enkephalin, serotonin, neurotensin, and paraaminoclonidine binding in 9 limbic thalamic nuclei and throughout cingulate cortex. Binding was assessed in the following thalamic nuclei: parvocellular and magnocellular divisions of the anterodorsal nucleus (ADp and ADm, respectively); parvocellular and magnocellular divisions of the anteroventral nucleus (AVp and AVm, respectively); anteromedial nucleus (AM); lateral magnocellular and laterodorsal nuclei (LM and LD, respectively); parvocellular and magnocellular divisions of the mediodorsal nucleus (MDp and MDm, respectively). Specific OXO-M/PZ binding increased progressively in AVp throughout training, reached peak levels at the criterial stage of performance and returned to control values during extinction training (Figure 1). The increase during criterial performance was significantly different than that for animals who were yoked to criterial performance. Thus, these changes in binding are likely due to discriminative learning processes because the training-induced progression in binding changes and the differences from yoked control cases. In ADp there was an increase in OXO-M/PZ binding early in training when the animals were first exposed to pairing of the conditional and unconditional stimuli, while that in the AVm nucleus occurred late in training during criterial performance. Neither of these latter changes were significantly elevated over the animals that were yoked to criterial performance, and binding was unaltered in any other thalamic nuclei.

A thorough analysis of OXO-M/PZ binding was made throughout the rostrocaudal extent of cingulate cortex. Specific OXO-M/PZ binding increased in most layers of rostral area 29c when subjects first performed a significant behavioral discrimination. Binding in more rostral and caudal levels of cingulate cortex was unchanged. The differences in rostral area 29c were significantly elevated over naive control cases. Although they were not significantly elevated over animals yoked to criterial performance, we are in need of cases which were yoked to first significant performance in order to have a valid comparison, i.e. it is not appropriate to compare animals yoked to criterial performance to those that were trained to the stage of first significant performance. OXO-M/PZ binding was also altered in area 29d, the dorsal division of cingulate cortex, but only in layer V. Specific binding of pirenzepine was unaltered in any thalamic or cortical area analyzed.

It is known from previous receptor localization studies (Vogt and Burns, 1988, J. Neurosci. 8: 643-652) that anterior thalamic lesions reduce OXO-M/PZ binding in cingulate cortex in a laminar pattern which is similar to that of the distribution of thalamic axon terminals in cingulate cortex. Therefore, it was hypothesized that training-induced increases in OXO-M/PZ binding in AVp might actually be responsible for changes in OXO-M/PZ binding in layer Ia of area 29c. There was a very high correlation between OXO-M/PZ binding in AVp and layer Ia of cingulate cortex as shown in Figure 2. This suggests that binding changes in cortex may be "driven" by anterior thalamic neurons.

Figure 2



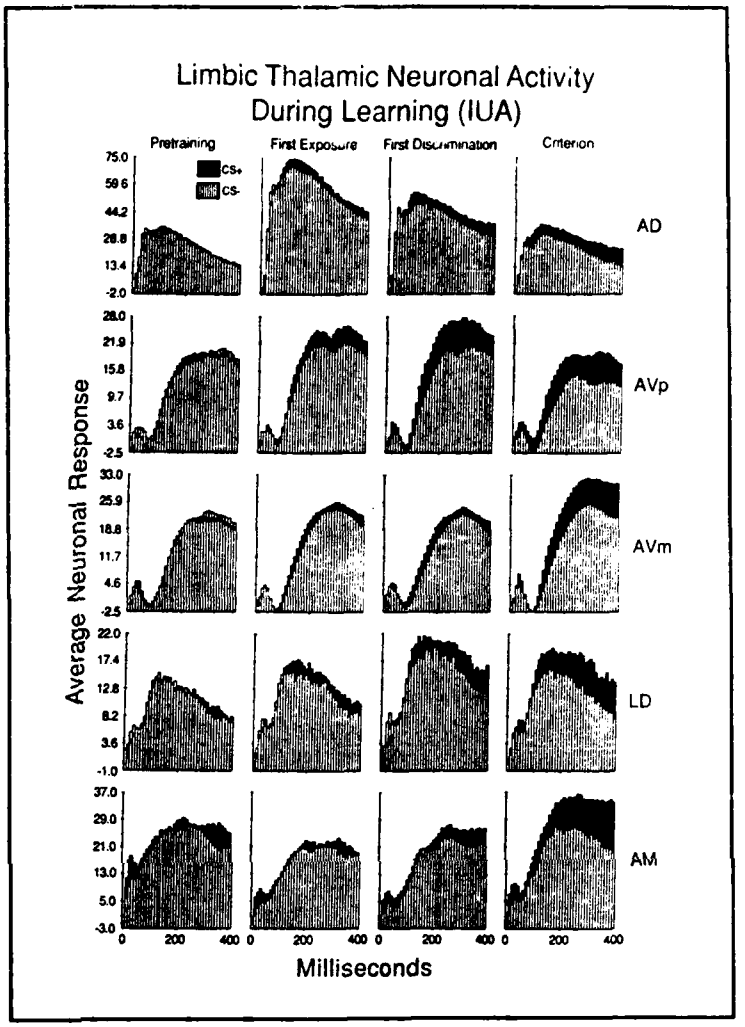
Increases in OXO-M/PZ binding but not pirenzepine binding suggests that binding to M_2 receptors is altered throughout discriminative avoidance training. It is possible that part of the change in cingulate cortex is associated with thalamic neurons because AV projects to layer I of area 29 and has neurons which synthesize M_2 receptors. Finally, since training-induced neuronal plasticities parallel changes in OXO-M/PZ binding, elevated M_2 binding may be a prerequisite for this activity in parts of the limbic system.

B. Neuronal Plasticities in Thalamus and Cortex

Gabriel, Vogt, Kubota, Poremba and Kang (1991) reported the findings of multi-unit recording studies which were conducted in 5 limbic thalamic nuclei and in layers of posterior cingulate areas 29c/d and 29b during the acquisition and performance of the discriminative avoidance task. Excitatory training-induced unit activity (TIA), i.e. increased tone-elicited activity during training relative to a pretraining session with unpaired tone-shock presentations, and/or discriminative TIA, i.e. greater discharges to the warning than to the safe tone, developed during training in 11 of the 13 areas analyzed. Discriminative TIA in the thalamic nuclei increased monotonically as learning progressed. As shown in Figure 3, AD and AVp excitatory TIA peaked early during the first session of training, LD excitatory TIA peaked in an intermediate stage when the first behavioral discrimination occurred and AVm and AM excitatory TIA peaked in a late stage of training when the first significant behavioral discrimination had occurred. The excitatory TIA in these nuclei declined as training continued beyond the stage in which peak activity occurred.

In cingulate cortex there were laminar differences as to when TIA occurred during the acquisition and performance of this task. Peaks of excitatory TIA developed in area 29c/d in the early (layer VI), intermediate (layers I-III and V) and late (layer IV) training stages as defined above. Only layer IV in area 29b of posterior cingulate cortex exhibited a peak of excitatory TIA which occurred in the early and intermediate training stages. As in limbic

Figure 3



thalamus, discriminative TIA increased monotonically over training stages in layers V and VI of area 29c/d and in layer VI of area 29b. However, layers I-III and IV in area 29c/d exhibited peak discriminative TIA in the intermediate and late training stages, respectively.

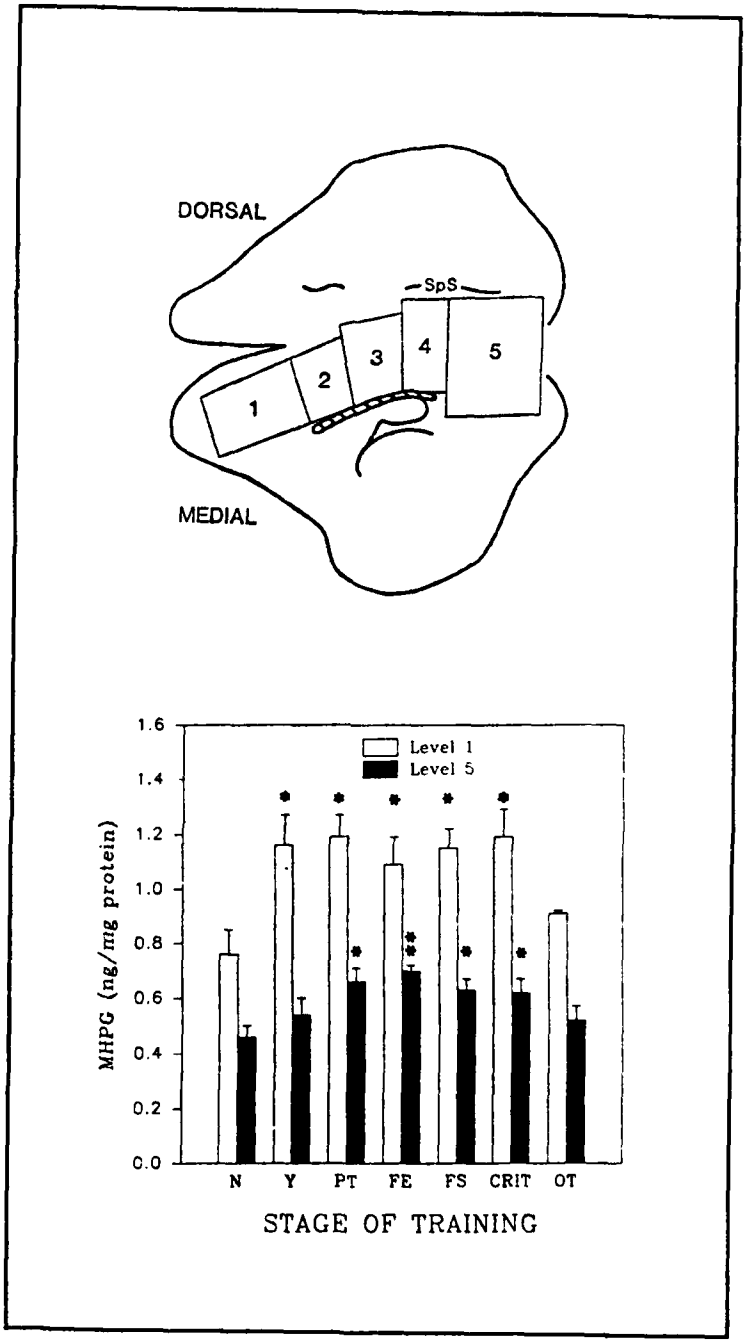
One interpretation of these findings is that the training-stage specificity of the thalamic and cortical excitatory peaks of TIA may reflect consolidation processes and provide a temporal label for the learned discrimination. Furthermore, it is important to notice that there are some relationships between alterations in OXO-M/PZ binding and TIA in the anterior thalamic nuclei. The increase in binding in ADp occurred during the first stage of training as did excitatory TIA in this nucleus. However, the binding remained high throughout training, while TIA was reduced with subsequent training. Specific binding of OXO-M/PZ in AVp peaked at the same stage as did discriminative neuronal activity during the session of first significant behavioral discrimination. Finally, binding in AVm peaked late in training during criterial stages of performance as was true for discriminative TIA in this nucleus.

C. Alterations in Thalamic $GABA_A$ & Opioid Receptor Binding

Specific muscimol binding to $GABA_A$ receptors was reduced in most limbic thalamic nuclei during first exposure to pairing of the positive conditional and unconditional stimuli (FE). This reduction was due to the conditioning contingency because there was no change in muscimol binding during pretraining when conditional stimuli and explicitly unpaired footshocks were presented. In addition, there were no differences between yoked and naive control groups and, with further training to criterial levels of performance, muscimol binding returned to control values. This pattern in binding was most pronounced in ADp where OXO-M/PZ binding was elevated during FE. The inverse regulation of binding to $GABA_A$ and M_2 receptors is an intriguing phenomenon both in terms of its behavioral significance and in terms of the mechanisms of receptor regulation. This inverse relationship will be explored further following unilateral or bilateral lesions of the lateral dorsal tegmental nucleus and either pretraining or training through FE, first significant or criterial performance.

Specific binding of (D-Ala^2-MePhe^4-Gly-ol^5)-enkephalin(DAGO) to mu opioid receptors or of a cyclic penicillamine containing enkephalin analogue (DPDPE) to delta opioid receptors was analyzed in 45 animals trained to different stages of performance and in 12 naive and 10 yoked control cases. Changes in DAGO binding were most pronounced in ADp, while those for DPDPE were most pronounced in the two divisions of AV. Specific DAGO binding in ADp fell from naive and yoked control values during pretraining and FE. Cases which received further training to first significant or criterial performance had control levels of DAGO binding. In contrast, binding of DPDPE was stable in ADp but was modulated during training in AVp and AVm. Thus, highest binding of DPDPE in these nuclei occurred during pretraining when the noxious footshocks were explicitly unpaired with one of the two conditional tones. Following FE and subsequent conditioning, levels of DPDPE binding returned to control values. Thus, this is another form of inverse changes in binding in the anterior thalamic nuclei. We propose to explore these changes further along with those in muscimol and OXO-M/PZ binding in the same cases which have received deafferentation lesions.

Figure 4



D. Noradrenaline Turnover During Training

Monoamines have been implicated in a number of neuronal plasticities including active avoidance conditioning. Lesions in cingulate cortex impair the acquisition and performance of discriminative avoidance learning and depletion of noradrenaline in posterior cingulate cortex impairs performance (Sparenborg and Gabriel, unpublished observations). The study by Vogt, Volicer, Schnepper and Gabriel (1991) employed high pressure liquid chromatography and a 16 electrode coulometric detection system to analyze the concentrations of noradrenaline, serotonin and dopamine and their principal metabolites in rabbit cingulate

cortex in subjects that had reached different levels of performance. Since cingulate cortex is not homogeneous in terms of the distribution of monoamines in its anterior and posterior cytoarchitectural divisions, measurements were made in tissue dissected from 5 rostrocaudal levels of cingulate cortex as shown in Figure 4.

Concentrations of dopamine and its metabolites were unchanged throughout training. The noradrenaline metabolite 3-methoxy, 4-hydroxyphenylglycol reached its highest concentration in posterior cingulate cortex during FE as shown in Figure 4. In anterior cingulate cortex the level of this noradrenaline metabolite was elevated during all stages of training including in yoked control cases in animals that were overtrained, i.e. given three days of training beyond criterial performance. The turnover of noradrenaline reached a peak in anterior cingulate cortex later in training when subjects first performed a significant behavioral discrimination. Turnover of serotonin was inversely related to that of noradrenaline in that the ratio of its metabolite 5-hydroxyindoleacetic acid to serotonin was highest in naive and overtrained animals when noradrenaline turnover was lowest. These data demonstrate that noradrenaline turnover progressively increases throughout the course of discriminative avoidance training and that these changes are not due to general arousal or stress. Training beyond criterial performance reduces noradrenaline turnover to basal levels.

E. Effects of Cingulate Lesions on Behavior and Neuronal Activity

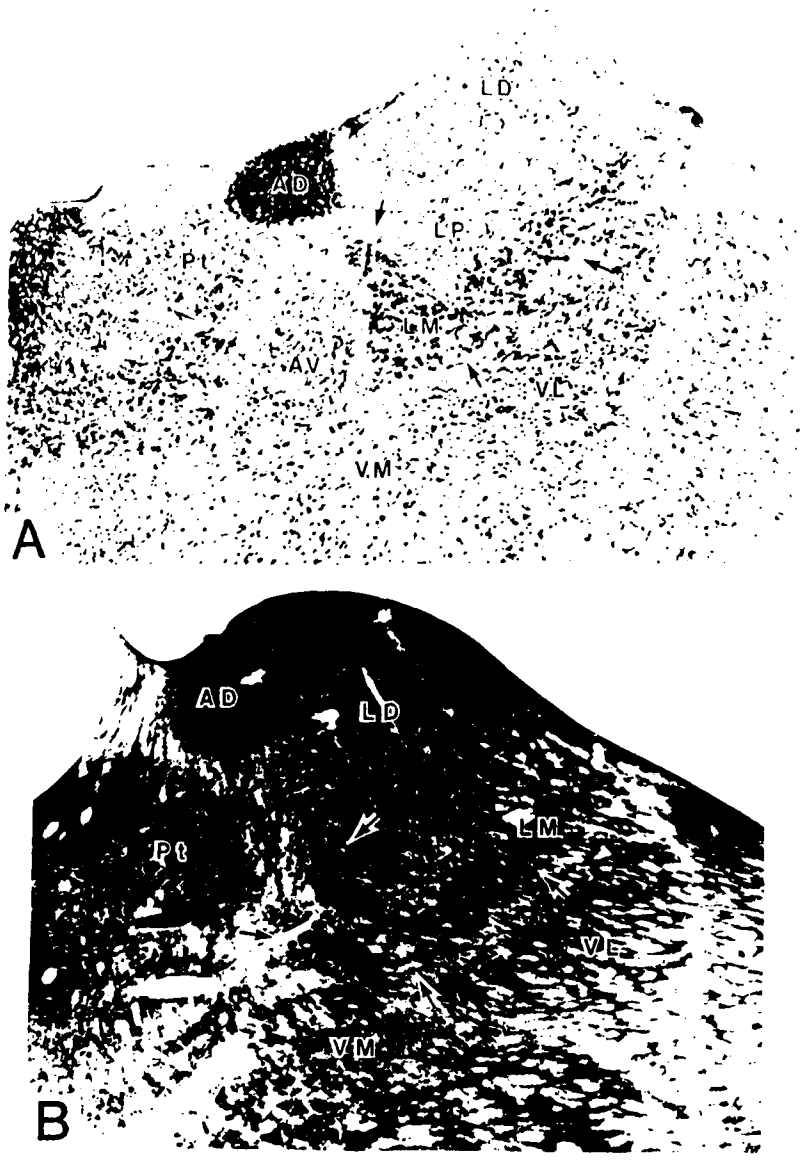
A study by Gabriel, Kubota, Straube and Vogt (1991) analyzes the role of anterior and posterior cingulate cortex in discriminative avoidance learning in rabbits as well as the influence of anterior cingulate lesions on training-induced neuronal activity in posterior cingulate cortex, mediodorsal thalamus and striatum. Rabbits with anterior and posterior cingulate cortex lesions took more than twice as many days to acquire this task than it did for control cases. Although there was only a mild retardation in acquisition of this task with ibotenic acid lesions in area 24, there was no evidence of "escape learning." This latter process refers to a progressive decrease in the latency of unconditioned responses which occurs during training in control animals. Ibotenic acid lesions in anterior cingulate cortex significantly increased positive conditional stimulus evoked neuronal activity during training but not pretraining sessions. These same lesions uncovered training-induced unit activity in the caudate nucleus during the stage of first significant behavioral discrimination and during criterial performance which was not present in the control cases. Finally, the anterior lesions removed early-developing, training-induced activity in posterior cingulate cortex, but they did not affect later-developing activity.

These data suggest that anterior cingulate cortex is crucial for processes that occur early in training. It has been postulated that anterior cingulate cortex, the laterobasal nucleus of the amygdala and mediodorsal thalamic nucleus are involved together in the "recency" or "working" memory system. The dependency of posterior cingulate cortex on anterior cingulate cortex function is clarified with the lesion and recording data and it can now be argued that the caudate nucleus is actually involved in discriminative neuronal processes.

F. Lateral Magnocellular Nucleus in Rabbit Thalamus

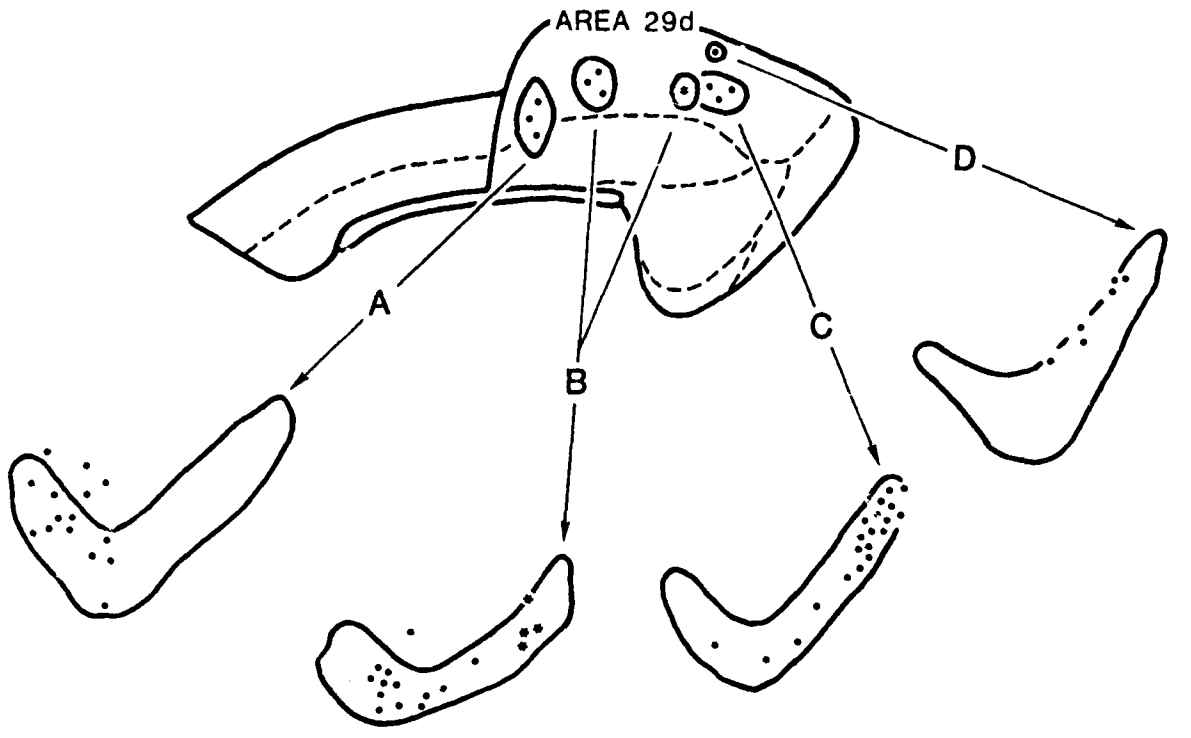
The lateral magnocellular nucleus (LM) contains the largest neurons in the rabbit thalamus, yet its cortical connections have not been detailed and nothing is known of its activity during the progression of discriminative avoidance training. The study by Vogt and Sikes (1990) evaluated the architecture, cingulate cortical connections and spontaneous rate of neuronal discharges in LM.

Figure 5



These micrographs are of transverse sections through the maximal rostrocaudal extent of LM in a celloidin embedded, cresyl Nissl stained case (A, 44×) and in a frozen section acetylcholinesterase reacted case (B, 44×). The borders of LM are marked with [illegible] The lack of LM is at the most caudal extensions of AV and AV [illegible] The large size of the AV stained [illegible] LM are apparent in A as is the high level of cytochrome [illegible] activity in B.

Figure 6



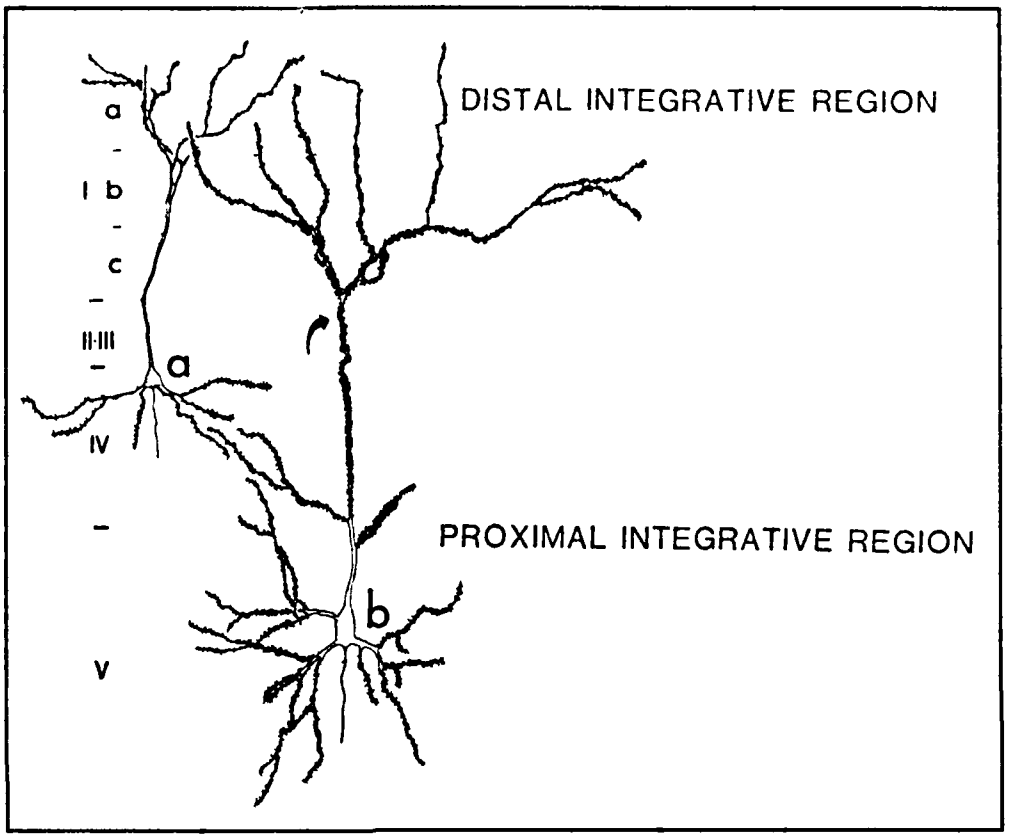
Injections were made into different rostrocaudal or mediolateral levels of area 29d with diamidino yellow (**A,B,D**), fast blue (B*), or horseradish peroxidase (**C**). Cytoarchitectural areas are displayed on a flattened medial surface above the corpus callosum and the level of LM is its maximal rostrocaudal extent as also presented in Figures 1, 4, and 5. Labeled neurons in LM were progressively more lateral in LM as the injections in area 29d were more posterior. In addition, the lateral injection in area 29d (D) labeled neurons in a dorsal part of LM when compared to more medial injections such as that in C.

At its maximal mediolateral extent in coronal sections, LM underlies the laterodorsal and lateroposterior nuclei. It has a short medial and long lateral limb, both of which have high levels of cytochrome oxidase activity as shown in Figure 5. On the basis of horseradish peroxidase and fluorescent dye injections, LM projects primarily to area 29 and posterior area 24. Projections to area 29d are topographically organized as shown in Figure 6. The medial limb of LM projects to rostral area 29d, mid levels of LM where the limbs join project to midlevels of area 29d and lateral parts of the lateral limb project to posterior area 29d. It is mainly the midportion of the lateral and medial limbs that projects to areas 29b and 29c. The anterior parts of these areas receive input from dorsal parts of LM, whereas posterior levels of these areas receive input from ventral LM. The midregion of LM also projects to caudal area 24. Injections of ^{3}H-amino acids into area 29d anterogradely labeled neuronal processes in LM. Finally, single unit electrophysiological recordings from LM in halothane-anesthetized rabbits showed a unique pattern of spontaneous discharges. Over 70% of the LM neurons cycled through a number of different phases from very high levels of 82 Hz to relatively low levels of 21 Hz.

Their size, high levels of cytochrome oxidase activity and spontaneous discharge rates suggest that LM neurons have a high level of metabolic activity and may share similarities to the centrolateral nucleus in other species. Furthermore, the extensive projections of LM to posterior cingulate cortex suggest that neurons in LM likely play a critical role in the functions of area 29.

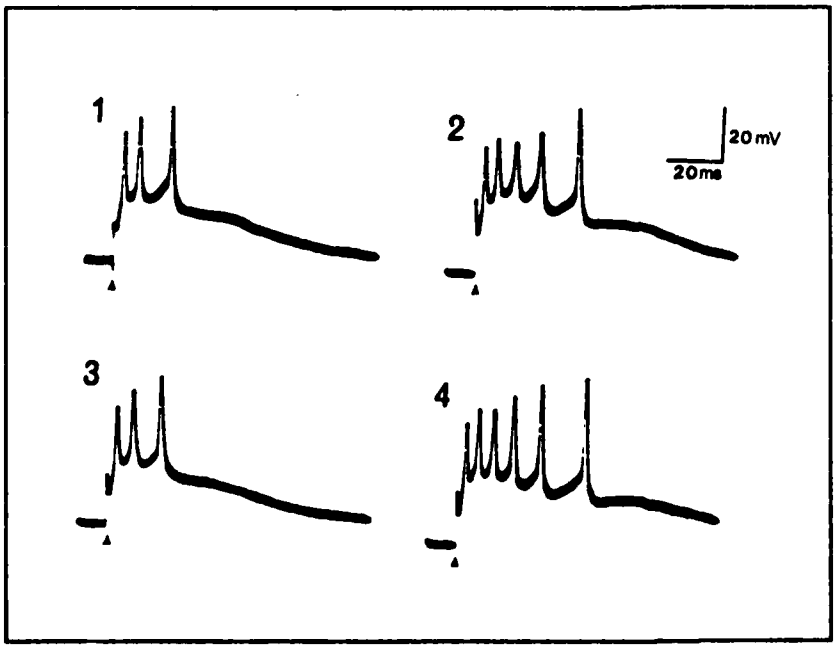
G. Layer I Structure and Function and Role in Memory

Figure 7



The review of layer I structure, connections and receptor binding is the first synthesis of its kind (Vogt, 1991) and provides hypotheses regarding the role of particular cortical afferents in parallel processing of sensory afferents, learning and memory and the consequences of its disruption in "primary subcortical gliosis." This analysis of layer I provides an important context in which to interpret changes in receptor binding throughout the course of discriminative avoidance training. The following premises were made: 1) Layer I function must be defined in terms of its primary components; apical dendritic tufts as shown in Figure 7 and afferent axons. 2) Apical dendritic tufts are a displaced integrative region in pyramidal neurons with a distinct morphology and GABAergic input. Inhibitory projections to layer I likely gate excitatory input from apical dendrites in layer I to those in deeper layers. 3) Disruption of layer I interferes with perceptual

Figure 8



processes and learning and memory. 4) Layer II neuron physiology likely reflects activity in layer I because the preponderance of its input is from layer I. Thus, afferent transmission through the midline and intralaminar thalamic nuclei to layer I in all sensory systems evoke activity which has a slow conduction velocity from the periphery and whose receptive field information is degraded along the course of central transmission. 5) Interactions between the distal and proximal integrative regions are assured by the following neuronal characteristics. a) Pyramidal neurons are electrotonically compact and so current is likely transmitted from apical tufts to somata in deeper layers. b) Calcium currents enhance transmission along apical dendrites. c) Cholinergic projections to both distal and proximal dendritic compartments further reduce the electrotonic length of dendrites and generally enhance neuronal excitability. This point is emphasized with our earlier work in the *in vitro* callosal slice preparation. As shown in Figure 8, application of acetylcholine to the slice in 2 and 4 amplified electrically-stimulated callosal-responses in cingulate cortical neurons both in terms of duration of excitatory postsynaptic potentials and the number of evoked action potentials when compared to evoked activity without acetylcholine in the bathing medium as shown in 1 and 2. 6) Layer I connections are ultimately involved in converting transient events into permanent memory via joint cholinergic and noradrenergic projections. Thus, when connections of this layer are disengaged, pyramidal neurons are capable of responding to transient sensory events, but are unable to parallel process information among a number of sensory areas and are unable to establish a permanent.

H. Organization and Functions of Cingulate Cortex

The P.I. and Dr. Gabriel have recently initiated publication of a volume titled "Organization and Functions of Cingulate Cortex and Limbic Thalamus." This volume is being written in order to consolidate a large body of information about the dorsal limbic circuit, i.e. cingulate cortex and the limbic thalamic, striatal, amygdalar, claustral, periaqueductal gray and other regions with which it is connected. As can be seen by the roster of chapters and contributing authors in Figure 9, there are three basic areas that will be covered. First, a comprehensive analysis of the cytoarchitecture, cytology and connections of rat, cat, monkey and human cingulate cortex will be presented in chapters 1 to 7. Second, in chapters 8 to 14 there will be an analysis of information processing in cingulate cortex. This will include the function of the medial pain system, mechanisms of reward, regulation of autonomic function the spatial and oculomotor properties of cingulate neurons, the cingulate motor regions and a model system analysis of discriminative avoidance learning. Third, in the last chapters we move from a consideration of the functions of cingulate cortex in animals to that in humans. Chapter 15 is a transitional chapter in which Dr. Olney will analyze the organization of the glutaminergic and cholinergioc systems and their role in certain forms of neurotoxicity. He will pose mechanisms by which limbic system disease may arise from dysfunctions in the glutaminergic and serotonergic systems. The final chapters will consider the role of cingulate cortex in human behavior including recent observations on the role of anterior cingulate cortex in "attention for action" as well as disruption of cingulate cortex structure and function in psychiatric disorders like schizophrenia and during aging as in Alzheimer's disease.

This volume will be the first of its kind. It will provide a broad neurobiological context within which future studies of the dorsal limbic circuit will be conducted. It has already induced new investigations among the collaborators and is expected to continue to encourage such collaborations over the next five years. Support by the Air Force Office of Scientific Research

Figure 9

ORGANIZATION AND FUNCTIONS OF CINGULATE CORTEX AND LIMBIC THALAMUS

B.A. Vogt and M. Gabriel, Editors

has been fundamental to the P.I.'s and Dr. Gabriel's efforts to bring this volume to fruition. The individual and joint projects proposed in the continuation applications by Drs. Vogt and Gabriel have in the past and will continue in the future to depend to a large extent on support from the Air Force Office of Scientific Research.

III. Publications Completed During October 1988-November 1990

Vogt, B.A., Gabriel, M., Vogt, L.J., Poremba, A., Jensen, E.L., Kubota, Y. and Kang, K. (1991) Muscarinic receptor binding increases in anterior thalamus and cingulate cortex during discriminative avoidance learning. J. Neuroscience, in press.

Gabriel, M., **Vogt, B.A.**, Kubota, Y., Poremba, A. and Kang, E. (1991) Training-stage related neuronal plasticity in limbic thalamus and cingulate cortex during learning in rabbits. J. Neuroscience, submitted.

Vogt, B.A., Volicer, L., Schnepper, P.W. and Gabriel, M. (1991) Elevated turnover of noradrenaline in cingulate cortex during discriminative avoidance learning. Experimental Brain Research, submitted.

Vogt, B.A. and Sikes, R.W. (1990) Lateral magnocellular thalamic nucleus in rabbits: Architecture and projections to cingulate cortex. J. Comparative Neurology 299: 64-74.

Vogt, B.A. (1991) The role of layer I in cortical function. In: Cerebral Cortex 9: 49-80, A. Peters and E.G. Jones (eds.), New York, Plenum Publishing.

Gabriel, M., Kubota, Y., Sparenborg, S., Straube, K., and **Vogt, B.A.** (1991) Effects of cingulate cortical lesions on avoidance learning and training-induced unit activity in rabbits, Experimental Brain Research, submitted.

We plan to publish a number of other studies including those of the binding of muscimol and opioid ligands in thalamus but it is somewhat premature to speculate on the specific subject matter of these publications. In addition, as outlined above, Drs. Vogt and Gabriel have firm commitments from 25 scientists to contribute to an 18 chapter volume.

Vogt, B.A. and Gabriel, M. (1992) Organization and Functions of Cingulate Cortex and Limbic Thalamus. Birkhauser Boston, a Springer-Verlag company, Boston, MA

IV. Participating Professionals

Dr. Brent A. Vogt: Principal Investigator, Ph.D., Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University

Dr. Michael Gabriel: Principal Investigator, Ph.D., Department of Psychology, University of Illinois

Dr. Linda J. Porrino: Co-investigator, Ph.D., Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University

Dr. Steven R. Childers: Co-investigator, Ph.D., Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University

Dr. Samual A. Deadwyler: Collaborator. Ph.D., Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University

Leslie J. Kromer Vogt: Laboratory Manager, M.A., Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University

Eugene L. Jensen: Research Assistant, B.A., Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University

Pamella W. Schnepper: HPLC-CD consultant, B.S., ESA Corp., Bedford, MA

Ladislav Volicer: Neuropharmacologist, M.D./Ph.D., Department of Pharmacology, Boston University School of Medicine

V. Other Progress and Accomplishments

The laboratory and three investigators (B.A. Vogt, L.J. Vogt and E.L. Jensen) moved from Boston University School of Medicine to Bowman Gray School of Medicine at the end of year 2. This move meant a complete dismantling and reconstruction of our research facilities and resubmission of this AFOSR grant for year 3. It took 4 months for us to complete this process. One of the principal reasons for making this move is that the new research environment in the Department of Physiology and Pharmacology is very conducive to continuation of behavioral research funded by the AFOSR. Dr. Samuel Deadwyler is an important player in the field of learning and memory and his interests in information processing and long-term potentiation in the hippocampal formation including subicular projections to cingulate cortex will dovetail well with the intent and future directions of the research embodied in our behavioral work. Dr. Linda Porrino is one of the world's leading experts in the use of metabolic markers to study brain function and Dr. Steven Childers is a receptor pharmacologist with whom we are collaborating to unravel the mechanisms by which binding to muscarinic and other receptors is regulated throughout the course of discriminative avoidance learning. There are many other investigators in our department who are involved in studying the mechanisms of reward (Dr. James E. Smith, Chairman) and the molecular biology of peptide synthesis and release (Drs. William Sonntag and Mariana Morris). This is an outstanding environment in which to continue the proposed studies.

Computerized behavioral training apparatus has now been installed at Bowman Gray School of Medicine and has been used to perform pilot studies which will be detailed in the renewal application. We have purchased new equipment with AFOSR and departmental funds so that mechanistic receptor studies can be completed. These studies include Scatchard analysis, *in situ* hybridization, immunohistochemistry, quantitative autoradiography, enzyme activity assays both on slides and in homogenized tissue. The equipment includes a) Hacker-Bright cryostat, b) Sorval RC5B superspeed centrifuge, c) Beckman ultracentrifuge, d) Revco ultralow freezer for brain storage, e) Brandell 48 chamber cell harvester for in vitro receptor binding assays and f) scintillation counter. Thus, in addition to the fine roster of colleagues at this institution, we have three times the space we had at Boston University and a full complement of equipment with which to perform the next generation of behavioral studies.